Part I Shiitake

Chapter 4

Shiitake Bag Cultivation

SUNFLOWER SEED HULLS

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The Flower that Faces the Sun

The sunflower plant (*Helianthus annuus* L.) is a native species to North America (Fig. 1). Spanish explorers carried it with them to Europe and eastern Asia. This widely adapted summer crop is now grown in many temperate regions in the world. It has a relatively short growing season of 90-100 days from planting to maturity. Sunflower heads consist of 1,000-2,000 individual flowers joined together by a receptacle base. A well-known sunflower characteristic is that the flowering heads track the movement of the sun, a phenomenon known as heliotropism.

This plant seed is mainly used to produce cooking oil. For this purpose, the seeds are mechanically husked and the hulls remain behind as an abundant agro-industrial waste (Fig. 2). Sunflower seed hulls have been marketed for specialty purposes such as poultry litter, fireplace logs and other high-fiber products, but these markets are limited. The seed hulls have been used sometimes as roughage for ruminants such as cows and sheep, but the high content of lignin makes it useless as animal food. More often the hulls are burned at the processing facility. Burying the hulls in the soil has also been tried. However, this practice is unsafe for the sanity of the fields because the hulls contain the plant pathogen fungus *Sclerotinia sclerotium*.

The disposition of huge amounts of this agro-industrial waste not only poses a problem to the environment because it degrades very slowly, but also an additional problem comes from its low density which requires more trucks to transport same weight of hulls, thus making its transportation very expensive.

Figure 1. Sunflower

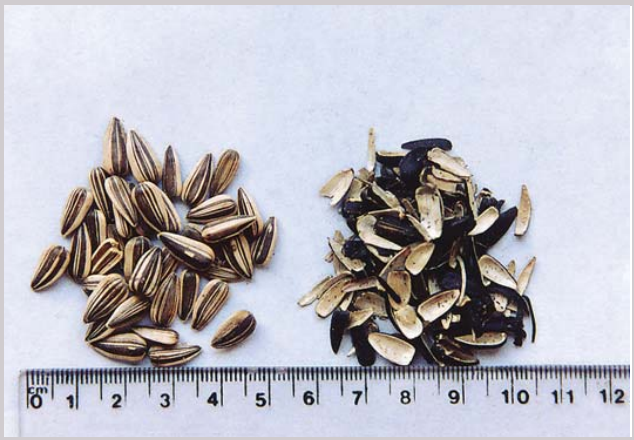

Figure 2. Sunflower whole seeds and hulls

The Composition of Sunflower Seed Hulls (SSH)

Sunflower seed hull (SSH) constitutes about 18-20% of the processed seeds (Helgeson *et al*., 1977). The main organic macro nutrients of SSH are lipids, carbohydrates and proteins, with the highest percentage of the content being in the lignin and cellulose-hemicellulose portion, with lignin comprising about 20-25% of the total weight (Dorrel and Vick, 1997). Reducing sugar[1] is also an important part of the seed coating. Total organic carbon coming from cellulose, hemicellulose and lignin account for more than 40%, making its C/N ratio quite high (Table 1). The lipids and protein contents are around 5% and 4%, respectively, and almost 3% of the lipids are waxes of long chain fatty acid and alcohols (Cancalon, 1971). Table 1 shows the approximate analysis and the mineral composition of the SSH produced by a local cooking oil industry (Bahia Blanca, Argentina). The embedding of the cellulose-hemicellulose portion in the lignin matrix makes the hulls highly stable in nature, accounting for its function in the seed: protection against water, thermal isolation and defense against pathogens.

This chemical composition of SSH makes it an attractive material for growing microorganisms, but the high lignin content limits the possibility of rapid biodegradation. The white rot fungi, basidiomycetes, are considered as the primary agents in nature for lignin degradation (Buswell and Oider, 1987).

Table 1. Approximate analysis of SSH

Humidity (%)	Total N (%)	Ash (%)	Total organic carbon (%)	Oxidable carbon (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)	C/N
11.8	0.58	3.0	42.0	11.7	28.7	31.3	25.2	72.4

Source: Santa María R., personal communication

Table 2. Mineral composition (g/kg) of SSH

P	K	S	Ca	Mg	Cu	Zn	Mn	Fe	Na	B	Pb
0.935	7.900	1.220	3.110	1.770	0.014	0.019	0.010	0.067	0.193	0.022	0.002

Source: Santa María R., personal communication

The biodegradation of lignocellulosic material by basidiomycetes is a cooperative process which involves the participation of oxygen reactive species (H_2O_2, superoxide and hydroxil radicals), other phenoxide radicals together with lignolytic enzymes collaborating with enzymes coming from the carbohydrate metabolism to degrade and assimilate the lignocelluloses (Leonowicz *et al*., 1999). In fact, shiitake (*Lentinula edodes*) produces enzymes capable of degrading cellulose and hemicellulose and oxidizing lignin (Buswell and Oider, 1987; Morais *et al*., 2000). It was therefore logical to launch experiments using sunflower seed hulls as an alternative substrate for shiitake cultivation.

Test on Shiitake Mycelial Colonization on SSH

The ability of shiitake to colonize substrates is limited by physico-chemical and nutritional factors (Kahn and Chaudhary, 1989; Song *et al*., 1987). So, the linear growth test (Duncan, 1997) of shiitake mycelium on SSH is first used in order to evaluate the mycelial colonization rate on different SSH substrate formulations containing different additives, looking at the removal of any limitation of growth because of the lacking of macro organic nutrients - mainly nitrogen - and eventually other major or minor inorganic nutrients or other growing factors like vitamins (Curvetto *et al*., 2002). Different formulations are prepared: SSH and wheat bran (9:1 and 8:2, by weight), SSH and poplar sawdust (9:1, by weight), and SSH, poplar sawdust, and wheat bran (8:1:1, by weight). All these substrate mixtures contained 37.5% lignocellulosic material- SSH, and the following salt and water levels: 0.5% $CaCO_3$, 2% $CaSO_4$ and 60% water, by weight. The substrate

Figure 3. Shiitake mycelium running on a tube containing SSH (linear growth test, Duncan, 1997)

[1] simple sugar - coming from the hydrolysis of the carbohydrate portion of the hulls - that contain aldehyde groups that are oxidised to carboxylic acids

was packed to an approximate density of 0.5g/cm³ in glass tubes 20cm long and 1.6cm diameter. An agar disk with shiitake mycelium was placed on one end of the tube. After plugging the tube ends with cotton, they were incubated at 25℃ in darkness for 12 days (Fig. 3).

The highest mycelial growth rates were obtained on substrates formulated with SSH and wheat bran (8:2) : 2.8mm/day, SSH and poplar sawdust (9:1) : 2.9mm/day, and SSH, wheat bran and poplar sawdust (8:1:1) : 2.9mm/day. For SSH alone it was 2.4mm/day, an interesting growth rate. The above results were good enough to proceed with the following step: to prepare some substrate formulations composed of mainly SSH to grow shiitake.

Shiitake Production on SSH Substrate

Spawn production

Spawn was prepared in 1 *l* bottles filled with a mixture of 59.1% wheat grain, 40% water, 0.1% $CaCO_3$, and 0.8% $CaSO_4$, by weight. Bottles were then sterilized at 15 psi for 90 minutes and were inoculated with *L. edodes* mycelium. The spawn was incubated at 25℃ in darkness for 15-20 days with periodic shakings.

Substrate preparation and spawn running

The simplest substrate formulation was used to evaluate the effectiveness of the additives on the fruiting yields, namely 37.5% SSH, 0.5% $CaCO_3$, 2% $CaSO_4$, 60% water and pH6. Since wheat bran is cheap and easily available, two additional formulations of SSH and wheat bran (8:2 and 9:1, by weight) were used.

Substrates were decontaminated following a technique previously developed in our laboratory (Curvetto *et al*., 1997, 2004), and were inoculated with shiitake spawn (7%, by weight) (Figs. 4). Bags were prepared as following; 1.5kg substrate was packed to an approximate density 0.5g/cm³ into 13 ×20cm polypropylene bags.

Bags were tightly closed and microperforations were made on their entire surface. Bags were then placed in a growth chamber at 24℃ and 70-80% R.H. for 25-30 days in darkness. Shiitake mycelium grew vegetatively until full colonization of the substrate and reached the "blistering" stage at 25-30 days from spawning.

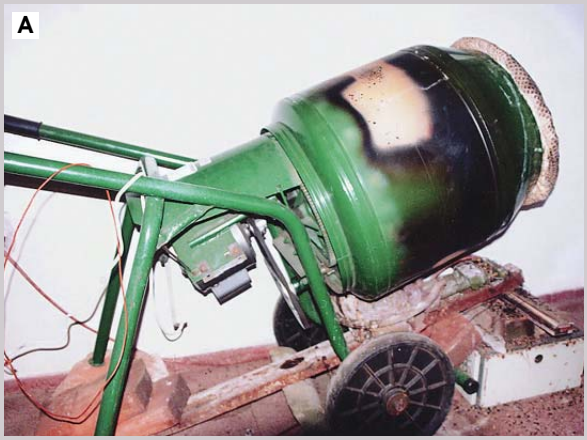

Figure 4. The substrate sterilization and inoculation **A**: A drum and gas heater for sterilization **B**: Putting sunflower seed hulls into the drum **C**: Spawning the decontaminated substrate after cooling **D**: Filling the bags

Fruiting, cropping and production

Figure 5. Shiitake cultivated on sunflower seed hull substrate

At pinning stage, the plastic bags were removed and the substrates were immersed in tap water at 4-6℃ in darkness for 48 hours, to initiate the fruiting stage. The substrates were kept in a controlled environment with 12 hours photoperiod under 1,500-2,000 lux, 80-90% R.H. 22℃ and adequate ventilation.

Fruiting was thus stimulated, and after 3-5 days mushrooms were ready for harvest. To obtain a second flush, synthetic logs were allowed to dry for 7-10 days in a room (at 50% R.H. and 25℃) and then the cold water treatment was repeated.

Yield parameters of shiitake mushroom grown on sunflower seed hull substrates are shown in Table 3. Caps appeared with typical shape and normal development, resulting in harvested mushrooms mainly with 6-8cm diameter (Fig. 5).

Table 3. Yield parameters of shiitake (Lentinula edodes) grown on different SSH substrate formulations in bags

Substrate formulation (by wt)	BE*(flush 1)	BE**(flush 2)	Accumulated BE	MP***	Productivity****
SSH	46 %	62 %	108 %	43 %	2.0 %
8 SSH : 2 wheat bran	49 %	63 %	112 %	47 %	2.0 %
9 SSH : 1 wheat bran	45 %	57 %	102 %	41 %	1.9 %

*BE : biological efficiency for the first flush at day 35 from spawn inoculation

**BE : biological efficiency for the second flush at day 55 from spawn inoculation

***MP: mushroom production at the second crop

****Productivity: mushroom production per day

It is concluded that the addition of wheat bran to the sunflower seed hull substrate did not produce significant differences in parameter yields or productivity. However, differences could occur in protein content and in the essential aminoacid index, both of which impact the nutritional index (Garcha *et al*., 1993), a parameter not evaluated in this study. The formulation containing only SSH as lignocellulosic material had a relatively high yield of 108% in 55 days. This represents a production rate of 2kg mushrooms from 100kg dry substrate per day, comparable to and even greater than that reported with other substrates based on hardwood sawdust, that have longer cultivation periods (Kalberer,1989; Pacumbaba and Pacumbaba, 1999a, 1999b; Przybylowicz and Donoghue, 1990; Rinker, 1991). Morais *et al*. (2000) obtained a BE of 60% after an approximately 100 days production cycle (a production rate of 0.6kg shiitake/100kg dry substrate per day). Hence, it appears that even though some nutrient deficiency was present in the substrate and culture conditions, it was not strongly limiting the shiitake growth performance.

In summary

Sunflower seed hulls can be used as a substrate for growing shiitake (*Lentinula edodes*), using the following formulation: 37.5% SSH, 0.5% calcium carbonate ($CaCO_3$), 2% calcium sulfate ($CaSO_4$), 60% water (H_2O) and pH 6. Under favorable conditions for mycelium growth, this material could be considered an adequate nutritional substrate for shiitake with no need of supplementation. However, formulations containing wheat bran could also be used. The plastic bag system using SSH as substrate produces higher yield of shiitake in a shorter cycle of production than with other substrates, i.e. substrates based on hardwood sawdust. A simple substrate formula like the one presented in this study, composed of an abundant and cheap residue from the cooking oil industry, would have a positive impact on production costs. By the time being, mushroom production is not already well established in the region. Our research institution is training future farmers through theoretical and practical courses in shiitake production using sunflower seed hull-based substrate.

REFERENCES

- Buswell, J.A., and O. Oider. 1987. Lignin biodegradation. *Critical Reviews in Biotechnology*. 6: 1-60.
- Cancalon, P. 1971. Chemical composition of sunflower seed hulls. *J. Amer. Oil Chem. Soc*. 48: 629-632.
- Curvetto, N., S. Delmastro, R. Devalis, and D. Figlas. 1997. A low cost method for decontaminating sunflower seed hull-based substrate in the cultivation of *Pleurotus* edible mushroom. *Mushroom Res*. 6(1): 25-28.
- Curvetto, N., D. Figlas, and S. Delmastro. 2002. Sunflower seed hulls as substrate for the cultivation of shiitake (*Lentinula edodes*) mushrooms. *Hort. Technology* 12 (4): 652-655.
- Curvetto, N.R., R. Gonzalez Matute, D. Figlas, and S. Delmastro. Cultivation of oyster mushrooms on sunflower seed hull substrate. In: *Mushroom Growers´ Handbook 1: Oyster Mushroom Cultivation*. Seoul, Korea: *MushWorld*. pp. 101-106.
- Dorrel, D.G., and B.A. Vick. 1997. Properties and process of oilseed sunflower. In: A.A. Schneiter ed: Sunflower technology and production. *Agronomy Monograph* 35: 709-745.
- Duncan, K.W. 1997. An ecophysiological approach to the evaluation, modification and production of mushroom growing media. *Mushroom News* 45(2): 12-27.
- Garcha, H.S., P.K. Khanna, and G.L. Soni. 1993. Nutritional importance of mushrooms. In: Chang, S.T., J.A. Buswell, and S.W. Chiu, eds: *Mushroom Biology and Mushroom Products*. Hong Kong: The Chinese University Press. Chapter 24.
- Helgeson D.L., D.W. Cobian, R.C. Coon, W.C. Hardie, L.W. Schaffner, and D.F. Scott. 1977. Dept. Agric. Econ., North Dakota Agric. Exp. Stat., North Dakota St. Univ. - Economic Development Administration. U.S. Dept. of Commerce, Washington, D.C. Bul. 503.
- Kalberer, P.P. 1989. The cultivation of shiitake on supplemented sawdust. In: *Proc. 12th Intl. Cong. Sci. Cultivation of Edible Fungi. Mushroom Sci*. χιι(ιι): 317-325.
- Kahn, S.M., and Y.A. Chaudhary. 1989. Some studies on oyster mushroom (*Pleurotus spp*.) on the waste material of corn industry in Pakistan. In: *Proceedings of the 12th International Congress on the Science Cultivation of Edible Fungi. Mushroom Sci*. χιι(ιι): 23-29.
- Leonowicz A., A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtas-Wasilewska, N.S. Cho, M. Hofrichter, and J. Rogalski. 1999. Biodegradation of lignin by white rot fungi. *Fungal Genetics and Biology* 27: 175-185.
- Morais, M.H., A.L. Ramos, N. Matos, and E.J. Santos Oliveira. 2000. Production of shiitake mushroom (*Lentinus edodes*) on lignocellulosic residues. *Food Sci. Technol. Intl*. 6(2): 123-128.
- Pacumbaba, R.P., and R.O. Pacumbaba. 1999a. Shiitake mushroom growth on formulated culture media, production of spawn, and basidiocarp in the laboratory. *Hort. Technology* 9(1): 85-90.
- Pacumbaba, R.P., and R.O. Pacumbaba. 1999b. Growing shiitake mushroom on hardwood sawdust in the greenhouse. *Hort. Technology* 9(1): 91-94.
- Przybylowicz, P., and J. Donoghue. 1990. Shiitake cultivation on sawdust. In: *Shiitake Growers Handbook: the Art and Science of Mushroom Cultivation*. Dubuque, Iowa: Kendall/Hunt Publishing Company. pp. 125-166.
- Rinker, D.L. 1991. The influence of heat treatment, genotype and other cultural practices on the production of shiitake mushrooms on sawdust. In: Maher ed: *Science and Cultivation of Edible Fungi*. Rotterdam, the Netherlands: A. A. Balkema. pp. 497-502.
- Song, C.H., K.Y. Cho, and N.G. Nair. 1987. A synthetic medium for the production of submerged cultures of *Lentinus edodes*. *Mycologia* 79: 866-876.